



GB04/01341



INVESTOR IN PEOPLE

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

The Patent Office
Concept House
Cardiff Road
Newport

South Wales
NP10 8EQ 17 MAY 2004

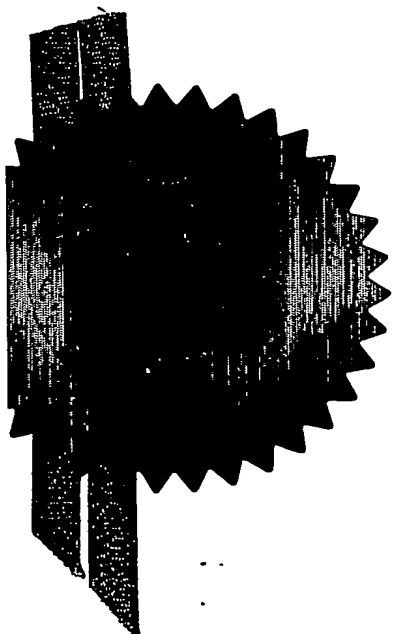
WIPO PCT

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



Signed

P. Mahoney

Dated

4 May 2004

Patents Act 1977
(Rule 16)

THE PATENT OFFICE

B

26 MAR 2003

NEWPORT



26MAR03 E795222-2 C95962
P01/7700 0.00-0306908.5

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference

AP 12

2. Patent application number
(The Patent Office will fill in this part)

23 MAR 2003

0306908.5

3. Full name, address and postcode of the or of each applicant (underline all surnames)

ANGIOGENE PHARMACEUTICALS LTD
MAGDALEN CENTRE
OXFORD SCIENCE PARK
OXFORD
OX4 4GA

GRAY LABORATORY
CANCER RESEARCH TRUST
PO BOX 100
MOUNT VERNON HOSPITAL
NORTHWOOD
MIDDLESEX
HA6 2JR

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

7244478003
SCOTLAND

4. Title of the invention

BIOREDUCTIVELY ACTIVATED STILBENE PRODRUGS

857622300

5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

ANGIOGENE PHARMACEUTICALS LTD
MAGDALEN CENTRE
OXFORD SCIENCE PARK
OXFORD OX4 4GA

Patents ADP number (if you know it)

7244478003

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

- a) any applicant named in part 3 is not an inventor, or
 - b) there is an inventor who is not named as an applicant, or
 - c) any named applicant is a corporate body.
- See note (d))

YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.
Do not count copies of the same document

Continuation sheets of this form

Description 16

Claim(s)

Abstract

Drawing(s)

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

11. I/We request the grant of a patent on the basis of this application.

Signature

Date

Peter D. Wardman 26/3/03

12. Name and daytime telephone number of person to contact in the United Kingdom

PETER DAVIS 01865 784660

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.
- b) Write your answers in capital letters using black ink or you may type them.
- c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- e) Once you have filled in the form you must remember to sign and date it.
- f) For details of the fee and ways to pay please contact the Patent Office.

BIOREDUCTIVELY ACTIVATED STILBENE PRODRUGS

This invention relates to compounds useful in the treatment of cell proliferation disorders.. More particularly the invention relates to a series of compounds that are activated under hypoxic conditions.

Many drugs used in conventional cancer chemotherapy are toxic to growing cancer cells but lack complete specificity. Thus other normal tissues are affected and ensuing side effects limit the dose that can be administered. Therefore the exposure of the cancerous tumour to the compound, and in turn the effectiveness of the therapy, is limited. Recent research has shown promising clinical activity of compounds, such as protein kinase inhibitors, which are cytostatic in their action. However the specificity of such compounds is not complete and side effects arising from action against normal tissues can again limit the effectiveness of therapy. There is a need for drugs that target the tumour more selectively.

Many solid tumours exhibit regions of hypoxia (low oxygen tension). Inadequate blood supply to the central regions of the tumour results in hypoxia that can be chronic or acute. This hypoxia represents a challenge to effective therapy by radiation or by conventional chemotherapy since hypoxic regions are often more resistant to these modalities. It has been suggested, however, that tumour hypoxia can be used to target tumours for drug action (Kennedy, *Cancer Res.* 1980, 40, 2356-2360.). One particular method of using the hypoxic regions of tumours for drug targeting is the selective activation of produgs under conditions of low oxygen tension. A concept has been advanced whereby the activity of a cytotoxic compound can be masked by a trigger moiety which, under hypoxic conditions, mediates fragmentation of the masked cytotoxic compound into the active cytotoxic agent (Denny, *Lancet Oncol* 2000, 1, 25-9). Compounds attempting to utilize this concept typically consist of the trigger moiety attached, often via a linker moiety, to a cytotoxic moiety (the effector).

Hypoxia is also a feature of the rheumatoid arthritic joint (Rothschild *Semin Arthritis Rheum* 1982, 12, 11-31). Cell proliferation is also a feature of the arthritic joint. Systemic antiproliferative drugs (for example methotrexate) are used in the therapy

of rheumatoid arthritis but are limited by side effects. Psoriatic lesions are also characterized by cell proliferation and hypoxia (Dvorak Int Arch Allergy Immunol. 1995, 107, 233-5).

A number of hypoxic trigger moieties have been disclosed including nitrobenzenes, nitronaphthalenes, nitroimidazoles, nitrofurans, nitrothiophenes, nitropyrroles, nitropyrazoles, benzoquinones, naphthoquinones, indoloquinones and azidobenzenes (for some examples see Naylor, Mini Rev. Med. Chem. 2001 1, 17-29; Tercel, J. Med. Chem. 2001, 44, 3511-3522 and Damen, Bioorg. Med. Chem. 2002, 10, 71-77).

A number of effector moieties have been utilised in the art including nitrogen mustards, phosphoramidate mustards, taxanes, enediynes and indole derivatives (for some examples see Naylor, *loc cit* and Papot, Curr. Med. Chem. Anti Cancer Agents 2002, 2, 155-185).

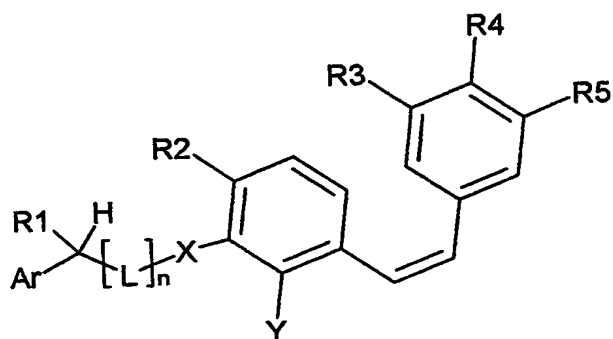
Hypoxic triggers joined to effectors via a linking group have been described wherein the linking group consists of a carbonate or carbamate (for some examples see Naylor, *loc cit* and Papot *loc cit*). In these cases it is intended that the intermediate carbonic acid or carbamic acid, formed by the initial hypoxia-driven fragmentation, further fragments to give the active agent.

The combretastatins are a series of stilbene compounds that have powerful antiproliferative activity against cancer cell lines *in vitro*. It is thought that this antiproliferative activity is due to an antimitotic action brought about by the inhibition of tubulin polymerisation. This antimitotic activity requires prolonged exposure of the cells to the compounds. Some of these compounds also have activity against tumour vasculature *in vivo*, which is distinct from the antimitotic activity observed *in vitro*. It is thought that the antimitotic activity of these compounds is generally not expressed *in vivo* because, unlike the antivascular activity, a prolonged exposure of the tumour to the compounds is required and the short elimination half-lives of the compounds preclude this exposure at non-toxic doses. Combretastatin analogues delivered to the tumour by a hypoxia-driven fragmentation strategy offer the potential to deliver prolonged tumour exposure that may be antimitotic while minimising host

toxicity. These compounds could also result in generation of active combretastatin compounds at the site of inflammation in rheumatoid arthritis and psoriasis, reducing the cell proliferation that is characteristic of these diseases.

It is an object of this invention to provide prodrugs that on bioreductive activation break down to release an antimitotic stilbene compound.

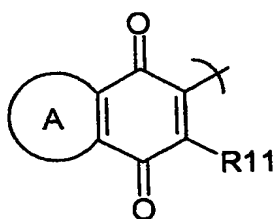
Thus according to one aspect of the invention we provide a compound of formula (1):



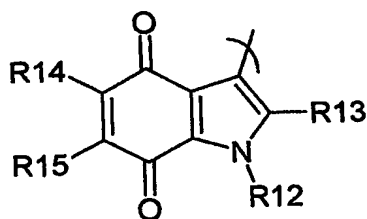
(1)

Wherein:

Ar is a substituted heteroaryl group bearing at least one nitro or azido group or is a group of formula (2) or (3)



(2)



(3)

R1 is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, aryl or COR6

R2 is alkyl, alkoxy, thioalkoxy or halo

R3, R4 and R5 are each independently alkyl, alkoxy, thioalkoxy or halo with the proviso that at least two of R3, R4 or R5 are alkoxy

L is -OC(O)- or -OP(O)(OR7)-

n is 0 or 1

X is O, S or NR8

Y is alkyl, alkoxy, thioalkoxy, halo, hydroxy or dihydrogenphosphate

R6 is OR8 or NR9R10

R7, R8, R9 and R10 are each independently H or alkyl

R11 is hydrogen, alkoxy or dialkylaminoalkyl

R12 is optionally substituted alkyl

R13 is hydrogen, alkyl, alkoxy or dialkylaminoalkyl

R14 and R15 are independently hydrogen, alkyl, alkoxy, thioalkoxy, amino, alkylamino, dialkylamino morpholino, alkylmorpholino, piperidino, alkylpiperidino, piperazino, alkylpiperazino or 1-aziridinyl

A is an optionally substituted aryl or heteroaryl ring

As used herein the term "alkyl", alone or in combinations, means a straight or branched-chain alkyl group containing from one to seven, preferably a maximum of four, carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl and pentyl .

An alkenyl group may be for example an olefinic group containing from two to seven carbon atoms for example methylene, ethylene, n-propylene, i-propylene, n-butylene, i-butylene, s-butylene and t-butylene. An alkynyl group may be for example an ethynyl , propynyl or butynyl group.

Optional substituents which may be present on alkyl, alkenyl or alkynyl groups include one or more substituents selected from halogen, amino, monoalkylamino, dialkylamino, hydroxy, alkoxy, alkylthio, alkylsulphonyl, aryl, heteroaryl, acylamino, alkoxycarbonylamino, alkanoyl, acyloxy, carboxy, sulphate or phosphate groups. The term "halogen" means fluorine, chlorine, bromine or iodine.

The term aryl means an unsubstituted phenyl group or a phenyl group carrying one or more, preferably one to three, substituents examples of which are halogen, optionally substituted alkyl, hydroxy, nitro, azido, cyano, amino and alkoxy.

The term heteroaryl is defined herein as a mono- or fused bi-cyclic aromatic group containing one to four heteroatoms selected in any combination from N, S or O atoms. Examples of heteroaryl groups include pyridyl, pyrimidyl, furyl, thienyl, pyrrolyl, pyrazolyl, indolyl, benzofuryl, benzothienyl, benzothiazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, quinolyl and isoquinolyl groups. A heteroaryl group can carry one or more, preferably one to three, substituents examples of which are halogen, optionally substituted alkyl, hydroxy, nitro, azido, cyano, amino and alkoxy.

Particularly useful values of the moiety Ar include nitroimidazole groups, for example 2-nitroimidazol-5-yl, and nitrothiophene groups, for example 5-nitrothien-2-yl.

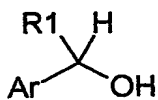
Where one or more functional groups in compounds of formula (1) are sufficiently basic or acidic the formation of salts is possible. Suitable salts include pharmaceutically acceptable salts for example acid addition salts including hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates, salts derived from inorganic bases including alkali metal salts such as sodium or potassium salts, alkaline earth metal salts such as magnesium or calcium salts, and salts derived from organic amines such as morpholine, piperidine or dimethylamine salts.

Those skilled in the art will recognise that compounds of formula (1) may exist as stereoisomers and/or geometrical isomers and accordingly the present invention includes all such isomers which have anticancer activity and mixtures thereof.

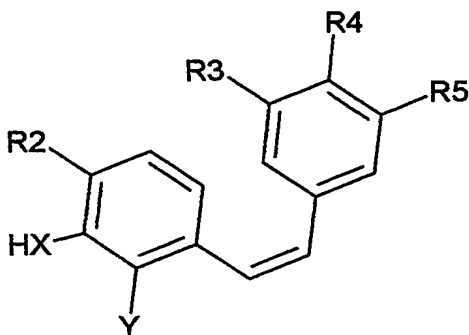
It is a further object of this invention to provide methods for the preparation of compounds of Formula (1).

Compounds of Formula (1) may be prepared by a number of processes as generally described below and more specifically in the Examples hereinafter. In the following process description, the symbols Ar, R1 and R2 when used in the formulae depicted are to be understood to represent those groups described above in relation to Formula (1) unless otherwise indicated. In the schemes described below it may be necessary to employ protecting groups that are then removed during the final stages of the synthesis. The appropriate use of such protecting groups and processes for their removal will be readily apparent to those skilled in the art.

Compounds of Formula (1) in which X is O or S and n is 0 can be prepared by Mitsunobu reaction of an alcohol of formula (4) with a stilbene of formula (5) in a solvent such as an ether solvent, for example tetrahydrofuran, diethyl ether or dioxan or in a solvent such as an aromatic hydrocarbon for example benzene or toluene or in a solvent such as an aprotic solvent for example dimethylformamide, in the presence of a phosphine for example triphenylphosphine or tri-*n*-butylphosphine and in the presence of an azo compound such as diethylazodicarboxylate, diisopropylazodicarboxylate or 1,1'-(azodicarbonyl)dipiperidine at a temperature from about 0°C to about the reflux temperature of the solvent, conveniently at room temperature.



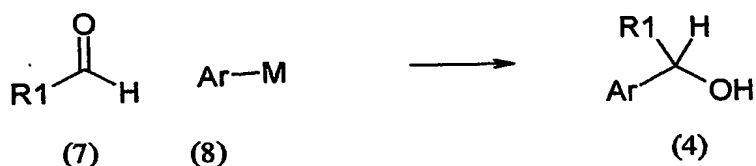
(4)



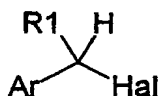
(5)

Alcohols of formula (4) are either known or can be prepared by standard methods apparent to one skilled in the art. Such methods include treatment of an aldehyde or ketone of formula (6) with a reducing agent, for example a borohydride reducing

agent such as sodium borohydride in a solvent such as an alcoholic solvent for example methanol at a temperature between about -20°C to room temperature, preferably around 0°C . Such methods also include the treatment of an aldehyde of formula (7) with an organometallic compound of formula (8) in which M represents a metal, metal halide or dialkylmetal, for example, Li, ZnBr, MgBr or MgI or dialkylaluminium in a solvent such as an ether solvent, for example tetrahydrofuran or diethyl ether or in an aromatic solvent for example benzene or toluene at a temperature of between about -78°C to about the reflux temperature of the solvent, preferably from about 0°C to room temperature. Where Ar is a substituted aryl or heteroaryl group bearing at least one nitro group such methods also include the aromatic electrophilic nitration of the appropriate aryl substrate with an appropriate nitrating agent at a temperature of between about -78°C and room temperature. Appropriate nitrating agents are, for example, nitric acid in a solvent such as an acid anhydride for example acetic anhydride or in a solvent such as an acid for example sulphuric acid or acetic acid; nitronium tetrafluoroborate in a solvent such as an ether solvent, for example tetrahydrofuran or diethyl ether or in a solvent such as acetonitrile or glacial acetic acid or in a solvent such as a chlorinated solvent for example dichloromethane or dinitrogen tetroxide in a solvent such as an ether solvent, for example tetrahydrofuran or diethyl ether or in a solvent such as acetonitrile or glacial acetic acid or in a solvent such as a chlorinated solvent for example dichloromethane or in an aromatic solvent for example benzene or toluene.

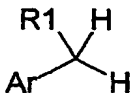


Compounds of formula (1) in which $n = 0$ can also be prepared by treatment of a halide of formula (9), in which Hal represents a chlorine, bromine or iodine atom, with a compound of formula (5), in a solvent such as an aprotic solvent such as dimethylformamide or in an ether solvent such as diethyl ether or tetrahydrofuran, or in a ketone solvent such as acetone in the presence of a base such as a metal carbonate for example potassium carbonate or silver(I)carbonate or a base such as a metal hydride for example sodium hydride or potassium hydride, at a temperature of between about -78°C to about the reflux temperature of the solvent preferably between 0° and room temperature.



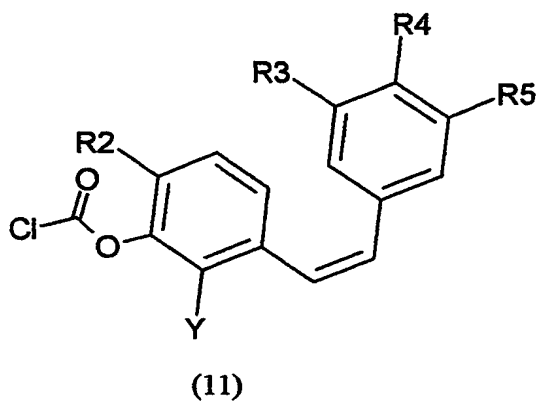
(9)

Halides of formula (9) are either known or can be prepared by standard methods apparent to one skilled in the art. Such methods include the halogenation of a compound of formula (10) with a halogenating agent such as N-bromosuccinimide, N-chlorosuccinimide or bromine in a solvent such as a chlorinated solvent for example dichloromethane or carbon tetrachloride at a temperature of about between about 0°C and the reflux temperature of the solvent.



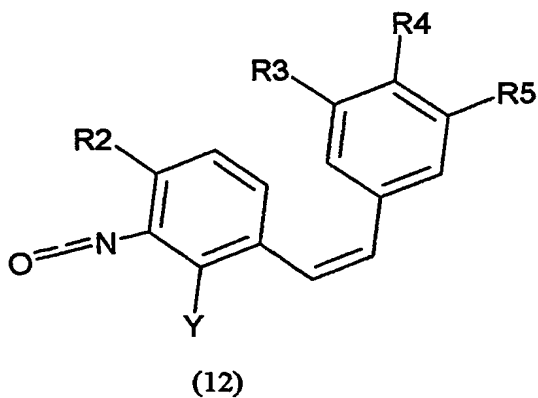
(10)

Compounds of Formula (1) in which X is O, n is 1 and L is $-\text{OC}(\text{O})-$ can be prepared by treatment of an alcohol of formula (4) with an acid chloride of formula (11) in a solvent such as a chlorinated solvent for example dichloromethane or trichloromethane at a temperature of between about 0°C and the reflux temperature of the solvent conveniently in the presence of a base such as, for example, an amine base for example pyridine or triethylamine.

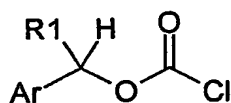


Acid chlorides of formula (11) are either known or can be prepared by standard methods apparent to one skilled in the art. Such methods include treatment of a compound of formula (5) in which $X=O$ with phosgene or triphosgene in a solvent such as a chlorinated solvent for example dichloromethane or trichloromethane with or without the addition of dimethylformamide at a temperature of around 0°C to room temperature.

Compounds of Formula (1) in which X is NH , n is 1 and L is $-\text{OC}(\text{O})-$ can be prepared by treatment of an alcohol of formula (4) with an isocyanate of formula (12) in a solvent such as a chlorinated solvent for example dichloromethane or trichloromethane at a temperature of between about 0°C and the reflux temperature of the solvent conveniently in the presence of a base such as, for example, an amine base for example pyridine or triethylamine.

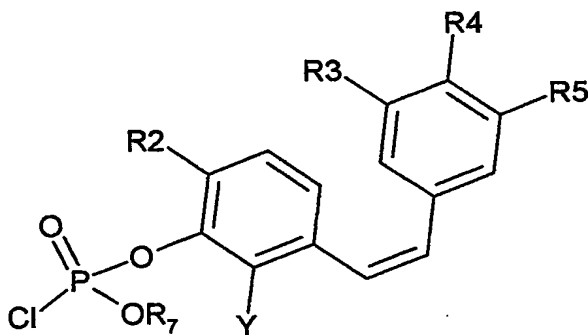


Compounds of formula (1) in which X is NR₈, n is 1 and L is -OC(O)- can be prepared by treatment of a chloroformate of formula 13 with a compound of the formula (5) in which X = NR₈ in a solvent such as a chlorinated solvent for example dichloromethane or trichloromethane at a temperature of between about 0°C and the reflux temperature of the solvent conveniently in the presence of a base such as, for example, an amine base for example pyridine or triethylamine.



(13)

Compounds of formula (1) in which n is 1 and L is -OP(O)(OR₇)- can be prepared by treatment of an alcohol of formula (4) with a compound of the formula (14) in a solvent such as a chlorinated solvent for example dichloromethane or trichloromethane at a temperature of between about 0°C and the reflux temperature of the solvent conveniently in the presence of a base such as, for example, an amine base for example pyridine or triethylamine.



(14)

Compounds of formula (1) can also be synthesized from other compounds of formula (1) by the application of standard methods, including substitution reactions, functional group transformations, bond-forming reactions and cyclisations known in the art.

Preparation of a compound of Formula (1) as a single enantiomer or, where appropriate, diastereomer may be effected by synthesis from an enantiomerically pure

starting material or intermediate or by resolution of the final product in a conventional manner.

The compounds of the invention may be administered as a sole therapy or in combination with other treatments. For the treatment of solid tumours compounds of the invention may be administered in combination with radiotherapy or in combination with other anti-tumour substances for example those selected from mitotic inhibitors, for example vinblastine, vincristine, vinorelbine, paclitaxel and docetaxel; alkylating agents, for example cisplatin, carboplatin, oxaliplatin, nitrogen mustard, melphalan, chlorambucil, busulphan and cyclophosphamide; antimetabolites, for example 5-fluorouracil, cytosine arabinoside, gemcitabine, capecitabine, methotrexate and hydroxyurea; intercalating agents for example adriamycin and bleomycin; enzymes, for example asparaginase; topoisomerase inhibitors for example etoposide, teniposide, topotecan and irinotecan; thymidylate synthase inhibitors for example raltitrexed; biological response modifiers for example interferon; antibodies for example edrecolomab and trastuzumab; receptor tyrosine kinase inhibitors for example gefitinib, and erlotinib; and anti-hormones for example tamoxifen. Such combination treatment may involve simultaneous or sequential application of the individual components of the treatment.

For the prophylaxis and treatment of disease the compounds according to the invention may be administered as pharmaceutical compositions selected with regard to the intended route of administration and standard pharmaceutical practice. Such pharmaceutical compositions may take a form suitable for oral, buccal, nasal, topical, rectal or parenteral administration and may be prepared in a conventional manner using conventional excipients. For example for oral administration the pharmaceutical compositions may take the form of tablets or capsules. For nasal administration or administration by inhalation the compounds may be conveniently delivered as a powder or in solution. Topical administration may be as an ointment or cream and rectal administration may be as a suppository. For parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) the composition may take the form of, for example, a sterile solution, suspension or emulsion.

The dose of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the compound chosen, the route of administration, the form and severity of the condition and whether the compound is to be administered alone or in combination with another drug. Thus the precise dose will be determined by the administering physician but in general daily dosages may be in the range 0.001 to 100mg/kg preferably 0.1 to 10mg/kg.

According to a further aspect of the invention there is provided a compound of formula (1), or a pharmaceutically acceptable salt or solvate thereof, for use in a method of treatment of the human or animal body by therapy.

A further feature of the present invention is a compound of formula (1), or a pharmaceutically acceptable salt or solvate thereof, for use as a medicament.

According to a further aspect of the invention there is provided the use of a compound of formula (1), or a pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament for use in the therapy of a warm-blooded animal, for example a human, suffering from a proliferative disease for example cancer.

The ability of compounds of the invention to release cytotoxic or cytostatic agents selectively under hypoxic conditions can be assessed by using, for example, one or more of the procedures set out below:

Radiolysis

In the hypoxic environments of solid tumours, prodrugs can be reduced by one-electron processes that are inhibited in the normoxic environments of normal tissues. Radiolysis demonstrates the ability of bioreductively-activated prodrugs to release the active drug after one-electron reduction. Compounds were dissolved in an isopropanol/water mixture (50:50) at a concentration of 50 μ M or below. Solutions, in gas-tight syringes, were saturated with nitrous oxide before irradiation in a ^{60}Co source at a dose rate of 3.9Gy min⁻¹ (as determined by Fricke dosimetry: H. Fricke and E.J. Hart, "Chemical Dosimetry" in Radiation Dosimetry Vol. 2 (F.H. Attrib and

W. C. Roesch. Eds.), pp 167-239. Academic Press New York, 1966.). Solutions were analysed for released drug by HPLC. In this test the compound of Example 1 produced combretastatin A4 with a radiation chemical yield (G-value) of $0.29 \mu\text{mol. J}^{-1}$.

Metabolism in tumour homogenates

Useful bioreductive prodrugs can be shown to release the active drug selectively under conditions of low oxygen in the presence of tumour homogenate in this assay. Freshly-excised CaNT tumours (approximately 0.5 to 1g) were homogenised in 15 ml of ice-cold 50 mmol dm^{-3} potassium phosphate buffer at pH 7.4. The homogenates were centrifuged at 1000 RPM for 10 min and the supernatants stored on ice. The metabolism of $5 \mu\text{mol dm}^{-3}$ prodrug in air and N_2 was performed with 0.5 ml tumour homogenate ($\sim 3 \text{ mg}$ of protein by Bradford assay) with $100 \mu\text{mol dm}^{-3}$ NADPH in 50 mmol dm^{-3} potassium phosphate buffer at pH 7.4 incubated at 37°C . Samples ($60 \mu\text{l}$) were taken at regular intervals and added to an equivalent volume of acetonitrile, then mixed and centrifuged at 14,300 RPM for 2 min prior to product analysis by HPLC. In this test the compound of Example 1 produced combretastatin A4 at a rate of $59 \text{ nmol. min}^{-1} \cdot \text{mg protein}^{-1}$ under nitrogen but only $7 \text{ nmol. min}^{-1} \cdot \text{mg protein}^{-1}$ under air.

Cellular Cytotoxicity

The cytotoxic or cytostatic properties of compounds of formula (1) and compounds of formula DrXH can be assessed for example, by use, for example, of this assay. The Celltiter 96[®] Aqueous One Solution Cell Proliferation Assay kit (Promega Corporation, USA) which is a colorimetric method for determining the number of viable cells in proliferation or cytotoxicity assays was used. In this assay the MTS tetrazolium compound (Owen's Reagent) is bioreduced by viable cells into a coloured formazan product which is soluble in tissue culture medium and can be measured by recording absorbance at 490 nm with a 96 well plate reader. A549 cells were seeded in Eagles Minimum Essential Medium supplemented with 10% foetal calf serum and non-essential amino acids at 10^3 cell per well on a 96 well plate and allowed to attach for

24 h. Compounds were dissolved in DMSO and diluted with cell culture medium before addition to The cells were exposed to test compound (0 to 2 $\mu\text{mol dm}^{-3}$) for 6 h then incubated for a further 72 h. The MTS reagent was added to each well, left for 4 h, then the absorbance measured at 490 nm with a 96 well plate reader.

The invention is illustrated by the following non-limiting Examples in which, unless otherwise stated:

DMF means dimethylformamide

THF means tetrahydrofuran

MeOH means methyl alcohol

EtOAc means ethyl acetate

DCM means dichloromethane

TLC means thin-layer chromatography

TFA means trifluoroacetic acid

MeCN means acetonitrile

Example 1

1-(4-Methoxy-3-(5-nitrothien-2-yl)methoxy)phenyl-2-(3,4,5-trimethoxy)phenyl-Z-ethene

5-Nitro-2-hydroxymethylthiophene (500 mg, 3.14 mmol) was dissolved in THF (5 ml) together with triphenylphosphine (1.68 g, 6.28 mmol) and combretastatin A4 (1.98 g, 6.28 mmol). To this solution was added diethylazodicarboxylate (1.09 g, 6.28 mmol) and the solution heated at 50 °C for 3 h, evaporated to dryness and the residue purified on silica (25% EtOAc/hexane) to give a pale yellow solid (mp 88-90°C, 810 mg, 57%) after recrystallisation from EtOAc/hexane. LC-RT 9.98 minutes (TFA 50-100%; MS (m/z, %) 457 (M^+ , 85 %), 316 (61 %), 301 (47 %), 252 (100%). Anal. C; 60.5, H; 5.1, N; 2.9 % $\text{C}_{23}\text{H}_{23}\text{NO}_7\text{S}$ requires C; 60.4, H; 5.1, N; 3.1 %.

Example 2**1-(4-Methoxy-3-(1-(5-nitrothien-2-yl)ethoxy))phenyl-2-(3,4,5-trimethoxy)phenyl-Z-ethene**

Diethylazodicarboxylate (357mg, 2.05mmol) was added dropwise to a solution of alcohol 2-(1-hydroxyethyl)-5-nitrothiophene (55mg, 0.32mmol), combretastatin A4 (648mg, 2.05mmol), triphenylphosphine (288mg, 1.10mmol) and THF (3mL). The reaction was stirred for 16 hours at ambient temperature and was then partitioned (EtOAc, brine), aqueous phase extracted (EtOAc), organic phase washed (H₂O, brine), dried (MgSO₄) and concentrated *in vacuo*. Flash chromatography, eluting sequentially with 50% EtOAc/hexane then 100% EtOAc, furnished the desired product as a yellow oil (15mg, 10%). LC-RT 3.85 minutes (100% MeCN). MS (m/z, %) 471 (M⁺), 425 (M⁺-NO₂), 316, 301, 141.

Example 3**1-(4-Methoxy-3-(5-nitrothien-2-yl)methoxycarbonyloxy)phenyl-2-(3,4,5-trimethoxy)phenyl-Z-ethene**

Phosgene (0.5 ml, 1 mmol) was dissolved in DCM (1 ml) at 0°C and under an argon atmosphere. To this solution was added combretastatin A4 (100 mg, 0.32 mmol) in DCM (0.5 ml) followed by triethylamine (60 µl, 0.4 mmol) after 1 h at 0°C. The solution was then stirred for 18 h while warming to 20°C, and then evaporated to dryness and re-dissolved in DCM (1 ml). To this was added triethylamine (56 ml, 0.4 mmol) and 5-nitro-2-hydroxymethylthiophene (60 mg, 0.376 mmol) and the solution stirred at 20°C for 5 days then evaporated to dryness. The residue was purified on silica (hexane/EtOAc, 4:1) to give a residue that was then purified by preparative HPLC (CH₃CN/H₂O, 9:1) to give 10 mg (7.5%) of a pale yellow gum. LC-RT 3.22 minutes (100% MeCN). MS (m/z, %) 501 (M⁺, 100 %), 316 (55 %), 301 (46 %), 252 (50 %).

Example 4

5-Methoxy-3-((3,4,4',5-tetramethoxy-(Z)-stilbene-3'-yl)oxy)methyl-1,2-dimethylindole-4,7-dione

Thionyl chloride (0.78 g, 6.5 mmol) was added drop-wise with stirring to a solution of 3-hydroxymethyl-5-methoxy-1,2-dimethylindole-4,7-dione (0.03 g, 0.13 mmol) in DCM (2 mL). The solution was stirred at 20°C for 1.5 h and evaporated to dryness. The residue was redissolved in DMF (1.5 mL) and potassium carbonate (0.054 g, 0.39 mmol) added, followed by combretastatin A4 (0.123 g, 0.39 mmol). The solution was stirred at 20°C for 18 h and EtOAc (50 mL) added. The solution was washed with water (25 mL), saturated sodium bicarbonate (25 mL) and brine (25 mL), dried (MgSO₄) and evaporated. The residue was purified by radial chromatography, eluting with EtOAc to give the title compound (15 mg, 21%) as an orange waxy solid: mp 192–194°C; LC-RT 5.13 minutes (80%MeCN). MS (m/z, %) 533 (M⁺, 3 %), 316 (27 %), 301 (24 %), 218 (100%). Anal. Fnd. C: 67.1, H; 5.9, N; 2.6% C₃₀H₃₁NO₈ requires C; 67.5, H; 5.9, N; 2.6%.

Example 5

3-((3,4,4',5-Tetramethoxy-(Z)-stilbene-3'-yl)oxy)methyl-1,2-dimethyl-5-(4-methylpiperazin-1-yl)indole-4,7-dione

The compound from Example 4 (53 mg, 0.1 mmol) was dissolved in anhydrous DMF (1.5 mL) together with 4-methylpiperazine (0.4 mL, ca. 4 mmol). The solution was stirred at room temperature for 48 h and then EtOAc (25 mL) added. The solution was washed with saturated sodium bicarbonate (25 mL) and brine (25 mL), dried (MgSO₄) and evaporated. The residue was purified on silica gel, eluting with 50% MeOH/EtOAc to give the title compound (28 mg, 47%) as a dark red gum. LC-RT 4.45 minutes (TFA 50–100 %). MS (m/z, %) 601 (M⁺, 7 %), 316 (26 %), 301 (19 %), 286 (100 %). Anal. Fnd. C: 60.6, H; 6.2, N; 6.1 % C₃₄H₃₉N₃O₇·4H₂O requires C; 60.6, H; 7.0, N; 6.2 %.

PCT/GB2004/001341

